REMARKS

Entry of this Amendment and reconsideration of the subject application in view thereof are respectfully requested.

I. Claim Status

Claims 1-58 were pending in the application. Of these, claims 3, 5, 18, 20, 30-35 and 41-58 were withdrawn, claims 1, 2, 4, 6-17, 19, 21-25, 28, 29, and 36-40 were rejected and claims 2, 17, 24, 26-28 and 37 were objected to. Claims 1, 4, 6, 7, 10, 11, 16, 19, 21-24, 28 and 37-39 have been amended to clarify the invention. No new matter is added.

II. Claim Objections

Claims 2, 17, 24, 26-28, and 37 were objected to because the claims recite limitations to non-elected inventions such as an antisense sequence or an antisense compound, a construct capable of expressing human L1RT antisense sequence, an inorganic compound, and peptide.

Applicant respectfully believes that no correction is required in response to the claim objections because the requirement for restriction with regard to the objected to claims 2, 17, 24, 26-28, and 37 (Group II claims) is predicated upon the nonallowability of linking claims. *See* the previous Office Action dated June 28, 2005 (Paper No./Mail Date 20050602). *See* also the notation on page 3 of the present Office Action stating that "claims 1 and 16 link inventions I-III. If these claims are held allowable the inventions will be rejoined, as explained in the previous Restriction Requirement." Pursuant to the rules of Patent Examining Procedure, "[w]here the requirement for restriction in an application is predicated upon the nonallowability of generic or other type of linking claims, applicant is entitled to retain in the application claims to the nonelected invention or inventions." MPEP §§ 809.03 and 821.01. Accordingly, Applicant respectfully believes that deletion of limitations directed to non-elected inventions is not warranted.

III. Rejections Under 35 USC § 112, Second Paragraph

Claims 4, 6, 7, 19, 21-23, and 28 stood rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject

matter which applicant regards as the invention.

Specifically, claims 4 and 19 were rejected based on the assertion that there are insufficient antecedent bases for terms "the nucleic acid" and "the DNA" recited in these claims. Claims 6, 7, 21, and 22 were rejected based on the assertion that there is insufficient antecedent basis for the term "the organic compound." Claim 23 was rejected based on the assertion that there is insufficient antecedent basis for the term "the cancer." There is insufficient antecedent basis for this limitation in the claim. Claim 28 was rejected under 35 U.S.C. § 112, second paragraph based on the assertion that the language of the claim is such that a person of ordinary skill in the art could not interpret the metes and bounds of the claim so as to understand how to avoid infringement (MEP § 2173.02).

Applicant respectfully believes that entry of the presently amended claims should overcome the rejections under 35 U.S.C. § 112, second paragraph of these claims. Accordingly, reconsideration and removal of the rejections are respectfully requested.

IV. Rejections Under 35 USC § 112, First Paragraph, Written Description

Claims 1, 2, 4, 6, 9, 10, 12-17, 19, 21, 23-25, 36, 37, 39, and 40 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Without conceding the validity of the rejection and solely to expedite the prosecution of the present patent application, Applicant has elected to limit method claims to the use of those inhibitors or antagonists indicated by the Examiner as complying with the written description requirement.

The Examiner further rejected claims 10-15, 39, and 40 as failing to comply with the written description requirement. Applicant respectfully traverses this rejection.

In rejecting these claims, the Examiner asserts that:

> "Applicants have not described the genus of methods encompassed by these claims because Applicants have not described cancers that are "due to" or "induced by" LINE-1 RT . . . The question is how does one determine that a particular cancer was induced by LINE-1 RT? Therefore, which cancers are treatable by the methods claimed in 10-15, 39 and 40? . . . Accordingly, Claims 10-15, 39, and 40 are rejected for failing to

> provide written description of methods for treating any and all cancers that are due to alternative lengthening of telomeres induced or mediated by LINE-1 RT, because no such cancers have been identified with reasonable clarity such that the skilled artisan would recognize that Applicants were in possession of the claimed methods."

Applicant respectfully disagrees with the Examiner's contentions and submits that patent disclosure as filed is sufficiently detailed to enable a person of skill in the art to recognize that the Applicant had invented the invention set forth in claims 10-15, 39, and 40. Specifically, the patent specification has sufficiently detailed description of relevant identifying characteristics, i.e., nucleic acid and amino acid sequence information for L1RT (*see* specification, for example, at page 6, lines 17-22 and at page 10 line 17 through page 11 line 2) and functional characteristics of L1RT discovered by the Applicant. For example, the specification at page 6, lines 2-24 describes that:

> "the present invention discloses that L1RT is involved in lengthening of telomeres in certain tumor tissues including telomerase negative tumors and the tumor-derived cell lines, and identifies L1RT enzyme or the sequences encoding it as a target for controlling the proliferative properties of the tumor cells or inducing apoptosis of these cells..
>
> The telomerase negative tumors and the tumor-derived cell lines are those that do not express or have the endogenous telomerase and yet show lengthening of telomeres, also referred to herein as alternative lengthening of telomeres (ALT). The L1RT mediated telomere lengthening in cells can be characterized by the presence of long and heterogeneous telomeres relative to the telomere lengthening mediated by telomerase. One skilled in the art would know how to determine the presence of long and heterogeneous telomeres characteristic of ALT in cells by carrying out, for example, TRF assay (*see*, Bryan et al., 1997, Nature Medicine, 3:1271-1274).
>
> ****
>
> In the present invention, it has been discovered that L1RT adds telomeric

DNA repeats to chromosomes in telomerase negative cells."

In addition, the specification also discloses test data demonstrating that the interference with L1RT activity or expression by an inhibitor or antagonist (e.g., AZT or an antisense sequence to L1 L1RT nucleic acid) induces progressive telomere loss, G2 phase arrest, chromosomal abnormalities and eventual cell death in telomerase negative osteosarcoma cancer cells. *See* Examples 1 and 2. The written description teaches that the invention can be applied to (detect, prevent and treat) L1RT mediated cancers, for example, osteosarcoma, breast carcinoma, ovarian carcinoma, lung carcinoma, adrenocortical carcinoma or melanoma. *See* the specification, for example, at page 6, line 25 through page 7, line 5 and at page 23, lines 12-16). In addition, the sequences for LINE-1 elements are known in the art, and the transcriptional activity of L1 has been demonstrated in a large number of tumors (*see*, for example, Kuo et al., 1998, Biochemical and Biophysical Research Communications, 253:566-570, a copy of which was included as part of the Information Disclosure Statement filed with the Patent Office on April 4, 2006). Based on such information, one of the skill in the art would know how to determine that a particular cancer was induced by LINE-1 RT and which cancers are treatable by the methods claimed in 10-15, 39 and 40.

Further, the patent specification is written for a person of skilled in the art, and such a person is deemed to come to the patent with the knowledge of what has come before. *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995). Applicant points out that it is not necessary to spell out every detail of the invention including every cancer treatable by the claimed methods in the specification to satisfy section 112, first paragraph. The written description requirement is that only enough must be included to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation. Therefore, Applicant respectfully submits that, after reading the patent application as filed, a person of skill in the art would understand how to make and use the methods and would understand the Applicant to have invented the methods set forth in claims 10-15, 39, and 40.

Accordingly, reconsideration and withdrawal of the written description rejections are respectfully requested.

V. Rejections Under 35 USC § 112, First Paragraph, Enablement

Claims 39 and 40 are rejected under 35 U.S.C. § 112, first paragraph, as failing to satisfy enablement requirement. Applicant respectfully traverses this rejection.

The Examiner essentially contends that:

> The instant claims are very broad. For instance, in their broadest embodiments, the claims encompass methods for prevention of any cancer using any nucleoside analog or combination of analog.

The enablement requirement of 35 U.S.C. § 112 is that the patent specification enable one of skill in the art to make and use the full scope of the claimed invention without "undue experimentation" as of the filing date of the application. *Chiron Corporation v. Genentech Inc.*, 363 F.3d 1247 (Fed. Cir. 2004); *AK Steel Corp. v. Sollac*, 344 F.3d 1234 (Fed. Cir. 2003). "The scope of patent claims must be less than or equal to the scope of the enablement. The scope of enablement, in turn, is that which is disclosed in the specification plus the scope of what would be known to one of ordinary skill in the art without undue experimentation. *Nat'l Recovery Techs., Inc. v. Magnetic Separation Sys., Inc.*, 166 F.3d 1190 (Fed. Cir. 1999).

At the outset, Applicant respectfully submits that the rejected claims are limited to prevention of such cancers that do not express telomerase but show L1RT mediated telomere lengthening in the cancer cells by using nucleoside analogs 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxyinosine (ddI) and 2',3'-didehydro-3'-deoxythymidine (d4T).

Regarding guidance, Applicant submits that the specification provides sufficient teachings to enable one skilled in the art to practice the invention set forth in claims at issue without undue experimentation. The specification, for example, at page 8, lines 3-10 teaches that:

> [t]he development of a cancerous tumor from a single immortalized cell or few such cells may take several months to years in humans. By practising the present invention, however, cancer can be prevented because the ability of the tumorigenic ALT cells treated with L1RT inhibitors lose their proliferative potential before they have had a chance to grow into a tumor. Further, periodic preventative administration of L1RT inhibitors or antagonists to at risk groups in order to stop tumor progression before

clinical manifestation of cancer could potentially decrease the rate of new cancer cases significantly.

The Example 1 demonstrates that treatment of the telomerase negative and L1RT expressing cells with AZT can induce progressive telomere loss, G2 phase arrest, chromosomal abnormalities and eventual cell death.

The *in vitro* models used in the present invention are acceptable models for demonstrating prevention of cancer in those animals including humans with one or more L1RT expressing tumorigenic cells before such cells have had a chance to grow into a tumor, and for demonstrating treatment of cancer. The guidance in the specification including the *in vitro* assays is sufficient to convince one of skill in the art of the asserted utility. Specifically, a person skilled in this art would recognize that the *in vitro* data in the specification as reasonably correlating to the claimed method and hence would have been able to practice the claimed invention by using only the teachings of the specification and the general knowledge available to such a person at the time the instant application was filed.

Given what is already described in the specification, determining the individuals in need of cancer prevention (e.g., at risk groups) or determining therapeutically effective amounts of a given nucleoside analog is not an inventive activity. Such findings would not place an undue burden on one skilled in the art, since it can be determined by routine and reasonable experimentation.

It may be that "numerous alterations" contribute to the emergence of a malignant cell, but once the malignant cell emerges it becomes immortal by stabilizing the length of its telomeres. This occurs through the activation of the enzyme telomerase or an alternative mechanism of telomere lengthening (ALT) (e.g., L1RT mediated telomere maintenance as in the instant case). As described above, the specification has sufficient teachings to enable one skilled in the art how to prevent such an immortal cell from becoming a tumor. The references (Bocchetta et al., 2004, Oncogene 23:6484-6491 and Chabner et al., 2005, Nature Reviews Cancer, 5:65-72) relied on by the examiner do not establish that cancer prevention by targeting telomere maintenance in cancerous cells is highly unpredictable.

Accordingly, Applicant respectfully requests reconsideration and removal of this rejection because the claims conform to the requirements of 35 U.S.C. § 112, ¶ 1.

VI. Rejections Under 35 USC § 102

Rideout

Claims 1, 2, 6, 7, and 9 are rejected under 35 U.S.C. § 102(b) as being anticipated by Rideout et al. (U.S. Pat. No. 5,683,990) ("Rideout"). This rejection is respectfully traversed and believed overcome in view of the following discussion:

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Schering Corporation v. Geneva Pharmaceuticals, Inc.*, 339 F.3d 1373 (Fed. Cir. 2003). Identity of invention requires that a prior reference disclose to one of ordinary skill in the art all elements and limitations of the patent claim. *Scripps Clinic v. Genentech*, 927 F.2d 1565, 1576 (Fed. Cir. 1991). Absence from the reference of any claimed element negates anticipation. *Kloster Speedsteel AB v. Crucible, Inc.*, 230 USPQ 81 (Fed. Cir. 1986). Inherent anticipation requires that the missing descriptive material is "necessarily present," not merely, probably or possibly present in the prior art reference. *In re Robertson*, 169 F.3d 743 (Fed. Cir. 1999). In general, a limitation or the entire claimed invention is inherent and in the public domain if it is the "natural result flowing from" the explicit disclosure of the prior art. *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 970 (Fed. Cir. 2001). To anticipate, the reference must also enable one of skill in the art to make and use the claimed invention. *In re Donohue,* 766 F.2d 531, (Fed. Cir. 1985).

Rideout is cited as teaching that "AZT and pharmaceutically acceptable salts thereof are . . . useful in treating Kaposi's sarcoma (KS) in human beings." Rideout is also cited as having clearly recognized "that HTLV III infected patients often develop cancers such as Kaposi's sarcoma and Epstein-Barr virus-related lymphomas."

However, the prophetic teaching in Rideout about the treatment of Kaposi's sarcoma does not anticipate the Applicant's invention and claims for at least the following reasons:

Applicant's invention is based on a finding that a cellular element viz., a reverse transcriptase encoded by L-1 (LINE-1) retrotransposon element adds telomeric DNA repeats to chromosomes to lengthen telomeres in telomerase negative tumors and the tumor-derived cell lines. Based on this finding, the invention identifies L1RT enzyme or the sequences encoding it as a target for controlling the proliferative properties of the tumor cells or inducing apoptosis of

these cells. The specification defines and discloses the inhibitors or antagonists of L1RT. *See* the specification at 7, lines 9-12, which states that

> The inhibitor(s) or antagonist(s) used in the present invention are those that directly or indirectly interact with L1RT to inhibit its expression (or activity) and/or those that get incorporated into telomere and thus prevent telomere from further elongation despite the functional L1RT thereby inhibiting the growth of cells expressing L1RT.

Each and every independent claim presently under examination in the instant application requires, among other things, a reverse transcriptase encoded by L-1 (LINE-1) retrotransposon element (or L1RT) and the defined inhibitor(s) or antagonist(s) of L1RT.

For example, the independent claim 1, as clarified, recites "[a] method of treating an individual suffering from a cancer characterized by cancer cells showing alternative lengthening of telomeres, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an inhibitor or antagonist of a reverse transcriptase, which reverse transcriptase is encoded by L-1 (LINE-1) retrotransposon and which is involved in said lengthening of telomeres in said cells of the individual, wherein the inhibitor or antagonist blocks said lengthening of telomeres, wherein the inhibitor or antagonist is a nucleoside analog 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxyinosine (ddI) or 2',3'-didehydro-3'-deoxythymidine (d4T).

Rideout simply teaches the use of AZT for treating Kaposi's sarcoma in human beings and does not teach or disclose each and every limitation as set forth in claim 1. Identity of invention requires that a prior reference disclose to one of ordinary skill in the art all elements and limitations of the patent claim. Applicant notes that, under the principles of inherency, if the natural result flowing from the performance of the Rideout's method would necessarily result in achievement of each of the claim limitations, then the claimed process is anticipated by the cited prior art reference. However, there is no evidence and the Examiner has not established that (i) Kaposi's sarcoma cells show alternative lengthening of telomeres; (ii) Kaposi's sarcoma cells express L1RT; and (iii) the natural result of AZT treatment in Kaposi's sarcoma patients is interference with such telomere lengthening in Kaposi's sarcoma cells.

In the absence of such evidence Rideout cannot anticipate the claims under the doctrine

of inherency for inherent anticipation requires that the missing descriptive material is "necessarily present," not merely, probably or possibly present in the prior art reference. As such the Examiner has not established a *prima facie* case of anticipation based on inherency.

Further, Applicant submits that the cancers (Kaposi's sarcoma and Epstein-Barr virus-related lymphomas) referred to in Rideout are virus-associated cancers seen in those patients infected with the AIDS virus. One skilled in the art knows that the HIV infection decreases antiviral cellular immune responses, increases the burden of persistent virus infections, and thereby predisposes HIV-infected people to develop virus-associated cancers, e.g., Kaposi's sarcoma. *See*, for example, Rideout at column 1 and Kieff, 1998, J. Natl Cancer Inst Monogr., 23:7-14 (a copy enclosed herewith as Exhibit 1). One skilled in the art also knows that the etiological agent in the case of virus-associated cancers is a virus and Kaposi's sarcoma is caused by herpesvirus. The natural result flowing from the disclosed Kaposi's sarcoma treatment is the repair of patients' immune system (e.g., T-helper cell activity) to the point where it is able to exert effective immune attack on persistent virus infection and the virus infected Kaposi's sarcoma cells to control and eventually destroy KS lesions. *See* Langford et al., 1989, Br J Dermatol. 120(5):709-713, a copy of which was included as part of the Information Disclosure Statement filed with the Patent Office on April 4, 2006.

In addition, it is known in the art that Herpes simplex virus encoded thymidine kinase, which can phosphorylate AZT, increases the available pool of phosphorylated AZT inside the infected cells. The high concentrations of phosphorylated AZT prevent normal cellular functions (e.g., inhibition of celluar kinases) and cause cell death due to cellular toxicity.

As such, given the strict identity required of the test for novelty, the Examiner has not established a *prima facie* case of anticipation in support of the rejection of claims 1, 2, 6, 7, and 9 based on the Rideout patent. Therefore, contrary to the Examiner's assertion, Rideout does not anticipate claims 1, 2, 6, 7, and 9 as it does not teach or disclose each and every limitation in each of these claims.

Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. § 102 (b) are respectfully requested.

Gan

Claims 16, 17, 21, 22-24, 29, 36-38 are rejected under 35 U.S.C. § 102(a) as being anticipated by Gan et al. (2002) FEBS Lett. 527:10-14 ("Gan"). Applicant respectfully traverses this rejection.

Claims 16, 24 and 37 are independent claims. Claim 16 is directed to a method of interfering with lengthening of telomeres in telomerase negative tumor cells. Claim 24 is directed to a method of preventing or inhibiting the growth of a telomerase negative cell showing alternative lengthening of telomeres. Claim 37 is directed to a method for interfering with L1RT activity in a system. All of these claims require the use of an inhibitor or antagonist (e.g. a nucleoside analog). The specification defines inhibitor(s) or antagonist(s), at page 7, lines 9-12, as follows:

> The inhibitor(s) or antagonist(s) used in the present invention are those that directly or indirectly interact with L1RT to inhibit its expression (or activity) and/or those that get incorporated into telomere and thus prevent telomere from further elongation despite the functional L1RT thereby inhibiting the growth of cells expressing L1RT.

Thus, by definition, the nucleoside analogs used in the present invention are those that get incorporated into telomere and prevent telomere from further elongation. Notwithstanding, Applicant has amended pertinent claims to clarify that particular inhibitors or antagonists are used to achieve a specific result (e.g., "the inhibitor or antagonist blocks lengthening of telomeres" in L1RT expressing cells).

Gan discloses the effect of AZT on telomere maintenance in telomerase negative Saos-2 cells. Gan is cited as anticipating claims 16, 17, 21, 22-24, 29, 36-38 for the reasons stated on pages 14-16 of the Office Action. In particular, the Examiner asserts that:

> [i]t is noted that the teachings of Gan et al. appear to contradict the teachings of the instant application. Namely, Gan et al. teach that AZT has no effect on telomere length in Saos-2 cells, whereas Applicants teach that AZT does affect telomere length in Saos-2 cells (*see* Example 1, page 30).
>
> Nevertheless, in view of the disclosure provided by Gan et al.

> showing the use of AZT in a telomerase-negative osteosarcoma cell line, the instant claims are anticipated by Gan et al.

Applicant respectfully disagrees and submits that Gan does not disclose each of the claimed limitations. For example, the claims require a specific result i.e., AZT prevent or block telomere from further elongation. Thus, the method claims herein require not only a telomerase negative cell and the use of AZT but also that the method achieve a specific claimed result. In contrast, as noted by the Examiner and based on the empirical evidence, Gan reports that AZT had no effect on the telomere length in Saos-2 cells. Therefore, Gan cannot anticipate, even under the principles of inherency, because the instant claims are limited by the very specific result (i.e., "the inhibitor or antagonist blocks lengthening of telomeres" in L1RT expressing cells) that the cited art failed to achieve. *See Bristol-Meyers Squibb Company v. Ben Venue Laboratories, Inc*, 246 F.3d 1368 (Fed. Cir. 2001).

Accordingly, reconsideration and withdrawal of this rejection based on Gan are respectfully requested.

VII. Rejections Under 35 U.S.C. § 102/103

Claims 10, 11, 13, 15-17, 21, 22, 24, 29, and 37-39 are rejected under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a), as obvious over Rideout et al. (U.S. Pat. No. 5,683,990) ("Rideout"). This rejection, which is allegedly based on inherency, is respectfully traversed and believed overcome in view of the following discussion:

Of the rejected claims, claims 10, 16, 24, 37 and 39 are independent claims. All of these claims are method claims and require inhibition of expression of L1RT or reverse transcriptase encoded by L-1 (LINE-1) retrotransposon.

The Examiner's bases for the concurrent rejections for anticipation and obviousness are as set forth on pages 17-18 of the Office Action. In particular, on page 18 of the Office Action, the Examiner notes that

> "Based on Applicant's teachings and those of the prior art, it cannot be presently determined whether Kaposi's sarcoma is a telomerase negative or telomerase positive tumor. Furthermore, it cannot be presently determined if KS is induced or mediated by LINE-1 RT. According to

> Applicant's teachings, there is a 30-50% chance that KS does satisfy these criteria. Thus, there is prima facie evidence to suggest that Rideout et al. teach the use of AZT to treat humans afflicted with a cancer that is induced or mediated by LINE-1 RT . . ."

The Examiner points to page 2 of the Applicant's specification in connection with the data concerning 30% and 50% of tumors as showing alternative lengthening of telomeres. The teachings on page 2 of the Applicant's specification concern background of the invention and the pertinent portion states the following:

> Elongation of shortened telomeres by telomerase is a well known mechanism of telomere maintenance in the human cancer cells. However up to 30% of human tumors of different types do not express telomerase. The presence of ALT was reported in up to 30% of human tumors of different types, tumor-derived cell lines and human cell lines immortalized *in vitro*[4,5,12,13], and up to 50% in some subsets of tumors and immortalized cell lines[14].

The references, numbered 4, 5, 12, 13 and 14, have been listed on pages 33-34 of the application and included as part of the Information Disclosure Statement filed with the Patent Office on April 4, 2006. As can be gleaned from these references, there is no mention of Kaposi's sarcoma, nor its telomerase negative status, nor LIRT activity in such cells. For example, the reference numbered 4 (Bryan et al., 1997, *Nat. Med.* 3, 1271-1274) reports ALT activity in melanoma osteosarcoma, breast carcinoma, ovarian carcinoma, lung carcinoma and adrenocortical carcinoma. The reference numbered 14 (Gupta et al., 1996, *J. Natl Cancer Institute,* 88, 1152-1157) reports that no telomerase activity was detected in 50% of the retinoblastomas. Kaposi's sarcoma is not retinoblastoma. Thus, the Applicant's teachings concerning "up to 30% of human tumors . . . and up to 50% in some subsets of tumors . . ." on page 2 of the specification refer to specific cancers other than Kaposi's sarcoma. Further, 50% chance for retinoblastoma to be a telomerase negative tumor does not necessarily translate into the same percentage chance for Kaposi's sarcoma.

Notwithstanding, inherency may not be established by chances, probabilities or possibilities, and there is no evidence that the inherent characteristics (i)-(iii) discussed above,

necessarily flow from the teachings of the Rideout patent.

The Examiner asserts that "[t]he basis for this rejection is found in MPEP § 2112." The explicit mandate of this section, however, is that "[t]he fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic . . . 'In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." In view of the above discussion, Applicant respectfully believes that the Examiner has not provided any objective evidence or cogent technical reasoning that could support the conclusion of inherency.

As pointed out by the Examiner, the MPEP does state "[T]he PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product" or "product-by-process claims." However, Applicant respectfully points out that the claims at issue are not product claims, nor product-by-process claims. Rather, the rejected claims are "process" claims. It does not claim AZT. It does claim, however, a process involving a new use for AZT, i.e., the use of AZT to block L1RT mediated telomere lengthening, which is an unknown use of AZT until the Applicant's discovery. Such claims are patentable. *See* MPEP 2112.02.

Accordingly, in contrary to the Examiner's assertion, no basis can be found in MPEP § 2112 for the rejection of claims 10, 11, 13, 15-17, 21, 22, 24, 29, and 37-39 under 35 U.S.C. 102(b) or under 35 U.S.C. 103(a) based on Rideout et al. Rideout does not teach or suggest the claimed methods. Reconsideration and withdrawal of the rejection are respectfully requested.

VIII. Rejections Under 35 U.S.C. § 103

Rideout and Mathias

Claims 1 and 4 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Rideout et al. (Pat. No. 5,683,990) ('Rideout") and Mathias et al., 1991, *Science* 254:1808-1810 ("Mathias"). Applicant respectfully traverses this rejection.

Rideout is discussed above. Mathias is cited as teaching LINE-1 elements. The Examiner contends that claims 1 and 4 are obvious for the reasons set forth on pages 20 and 21 of the Office Action. Essentially, relying on the principles of inherency, the Examiner asserts

> "[t]hough the skilled artisan may not have recognized at the time the invention was made that AZT was inhibiting LINE-1 RT, encoded by RNA transcribed from DNA, the artisan would not have needed to, since the inhibitory effects of AZT were inherently present."

Under the principles of inherency, however, the prior art must necessarily function in accordance with or include the claimed limitations. Inherency cannot be established by probabilities or possibilities. Specifically, for example, inherency cannot be established by an assertion that there is a 30-50% chance that Kaposi's sarcoma is a telomerase negative or telomerase positive tumor and KS is induced or mediated by LINE-1 RT. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. There is no evidence and the Examiner has not established that (i) Kaposi's sarcoma cells show alternative lengthening of telomeres; (ii) Kaposi's sarcoma cells express L1RT; and (iii) the natural result of AZT treatment in Kaposi's sarcoma patients is interference with such telomere lengthening in Kaposi's sarcoma cells. Notwithstanding, Applicant submits herewith evidence showing that the natural result flowing from the disclosed Kaposi's sarcoma treatment is the repair of patients' immune system (T-helper cell activity) to the point where it is able to exert effective immune attack on persistent virus infection and the virus infected Kaposi's sarcoma cells to control and eventually destroy KS lesions (*see* Langford et al., 1989, Br J Dermatol. 120(5):709-713, a copy of which was included as part of the Information Disclosure Statement filed with the Patent Office on April 4, 2006). Accordingly, the inhibitory effects of AZT cannot be said to be inherently present based on the disclosed Kaposi's sarcoma treatment.

Based on the alleged inherency, the Examiner further asserts that

> "[t}he skilled artisan would have been motivated to use AZT according to the method taught by Rideout et al. to treat an individual suffering from cancer . . . Similarly, the skilled artisan would have had a reasonable expectation of success given that Rideout et al. teach methods for administering AZT to humans, and given that Rideout et al. teach specific pharmaceutical formulations of AZT for use in treating humans."

Applicant respectfully submits that Rideout does not teach or suggest the method as set forth in claim 1. For example, Rideout does not teach or suggest that AZT blocks L1RT

mediated telomere elongation. Mathias fails to remedy the shortcomings of Rideout. Mathias, which concerns human LINE-1 elements, teaches that ORF2 from a human L1 element encodes a reverse transcriptase activity. Mathias does not teach or suggest the claimed method. For example, Mathias does not teach or suggest that AZT blocks L1RT mediated telomere elongation. To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. Therefore, even if the teachings of the cited references are combined, one cannot arrive at the claimed invention because the combination simply does not suggest all the claim limitations set forth in claim 1.

Further, it is well known in the art that AZT, which was originally intended to treat cancer, failed as a cancer treatment and had a dramatic second life as a treatment for HIV infection (failing initially as monotherapy, but succeeding in combination with other drugs). Indeed, there is no motivation to use AZT to treat an individual suffering from cancer characterized by cells of the cancer showing alternative lengthening of telomeres, and in so using there is no reasonable expectation of success. This is because AZT has been reported to have no effect on the telomere length in Saos-2 cells (*see* Gan et al., 2002, FEBS Lett. 527:10-14). Nevertheless, Applicant proceeded against this art accepted wisdom, and achieved telomere shortening in telomerase negative cells and their growth inhibition. Proceeding contrary to accepted wisdom in the art is evidence of nonobviousness.

Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness of independent claims 1 and 19 under 35 U.S.C. § 103(a). Further, the rejected dependent claim 4 by virtue of its dependency from the independent claim 1 is similarly considered by Applicant to patentably define itself over the cited references. As such, claims 1 and 4 stand in condition for allowance for these very same reasons. Reconsideration and withdrawal of this rejection are respectfully requested.

Rideout and Wagner

Claims 1 and 8 are rejected 35 U.S.C. 103(a) as being unpatentable over Rideout et al. (Pat. No. 5,683,990) ("Rideout") and Wagner et al. (1997) *Cancer Res.* 57:2341-2345 ("Wagner"). Applicant respectfully traverses this rejection.

Rideout does not teach or suggest the invention in claim 1 at least for the reasons

discussed above. For example, Rideout does not teach or suggest that AZT blocks L1RT mediated telomere elongation. Wagner fails to remedy the shortcomings of Rideout. Specifically, Wagner discloses that AZT inhibits the growth of the human breast cancer cell line, MCF-7 and the growth of MNU-induced rat mammary tumors. However, Wagner does not teach or suggest the claimed method. For example, Wagner does not teach or suggest that AZT blocks L1RT mediated telomere elongation. To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. Therefore, even if the teachings of the cited references are combined, one cannot arrive at the claimed invention because the combination simply does not suggest the method set forth in claim 1.

Further, there is no motivation to use AZT to treat an individual suffering from cancer characterized by cells of the cancer showing alternative lengthening of telomeres, and in so using there is no reasonable expectation of success because AZT has been reported to have no effect on the telomere length in Saos-2 cells and has been reported to be carcinogenic in mammals including humans.

Accordingly, Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness of independent claims 1 and 19 under 35 U.S.C. § 103(a). Further, the rejected dependent claim 8 by virtue of its dependency from the independent claim 1 is similarly considered by Applicant to patentably define itself over the cited references. As such, claims 1 and 8 stand in condition for allowance for these very same reasons. Reconsideration and withdrawal of this rejection are respectfully requested.

Gan and Mathias

Claims 16 and 19 are rejected under 35 U.S.C. § 103(a) as being patentable over Gan et al. (2002) FEBS Lett. 527:10-14 ("Gan"); and Mathias et al. (1991) *Science* 254:1808-1810 ("Mathias"). Applicant respectfully traverses this rejection.

Claim 16 is an independent claim. It requires, among other things, the inhibitor or antagonist must block lengthening of telomeres.

Gan is discussed above. Gan reports that AZT had no effect on the telomere length in Saos-2 cells (telomerase-negative cells) (*see* Gan et al., 2002, FEBS Lett. 527:10-14 at 13). Gan does not teach or suggest a method that achieves a specific result, i.e., the inhibitor or antagonist

blocks lengthening of telomeres in telomerase negative tumor cells. Mathias fails to remedy the shortcomings of Gan. Mathias, which concerns human LINE-1 elements, teaches that ORF2 from a human L1 element encodes a reverse transcriptase activity. Mathias does not teach or suggest the claimed method. For example, Mathias does not teach or suggest that AZT blocks L1RT mediated telomere elongation. To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. Therefore, even if the teachings of the cited references are combined, one cannot arrive at the claimed invention because the combination simply does not suggest all the claim limitations set forth in claim 1.

The Examiner purports to establish motivation and reasonable expectation of success, in view of Gan and Mathias, as follows:

> "[t]he skilled artisan would have been motivated to use AZT according to the method taught by Gan et al. as a research tool to inhibit the lengthening of telomeres in telomerase-positive cell lines and, because Gan et al. teach that AZT can be used to inhibit telomere lengthening in some cell lines.
>
> Similarly, the skilled artisan would have had a reasonable expectation of success given that Gan et al. teach methods for preparing, administering, and assaying AZT in cultured cell lines." (emphasis added).

However, there is no motivation to use AZT to inhibit the lengthening of telomeres in telomerase-negative cell lines, and in so using there is no reasonable expectation of success. This is because AZT has been reported to have had no effect on the telomere length in telomerase-negative cells.

Further, Gan's disclosure discredits or otherwise discourages the solution claimed by its explicit teaching that AZT had no effect on the telomere length in Saos-2 cells (telomerase-negative cells). Nevertheless, Applicant proceeded against this art accepted wisdom, and achieved prevention of telomere elongation in telomerase negative cells and their growth inhibition. Proceeding contrary to accepted wisdom in the art is evidence of nonobviousness.

Thus, Applicant respectfully submits that the Examiner has not established a *prima facie* case of obviousness of independent claim 16 under 35 U.S.C. § 103(a). Further, the rejected dependent claim 19 by virtue of its dependency from the independent claim 16 is similarly

considered by Applicant to patentably define itself over the cited references. As such, claims 16 and 19 stand in condition for allowance for these very same reasons. Reconsideration and withdrawal of this rejection are respectfully requested.

Gan

Claims 16 and 25 are rejected under 35 U.S.C. 103(a) as being patentable over Gan et al. (2002) FEBS Lett. 527:10-14 ("Gan"). Applicant respectfully traverses this rejection.

Gan as applied to claim 16 is discussed above. Claim 25, which depends from claim 24, recites that "the cell is contacted with a nucleoside analog at a concentration of 0.2 μM." Claim 25 also requires that the nucleoside analog must block lengthening of telomeres in a telomerase negative cell.

Gan reports that AZT had no effect on the telomere length in Saos-2 cells (telomerase-negative cells) treated with AZT at 0, 1 and 10 μM (*see* Gan et al., 2002, FEBS Lett. 527:10-14 at 13 and Figure 4). Without conceding the validity of the Examiner's assertions as to "Overlap of Ranges," Applicant respectfully submits that there is no motivation given to one skilled in the art to make the claimed invention as a whole, i.e., to select 0.2 μM AZT from the disclosed prior art range, given the reference's express teachings that AZT had no effect on the telomere length in telomerase-negative cells. There is no reasonable expectation that the range of AZT tested by Gan, once modified to include 0.2 μM AZT would be successful. In fact, the expectation would most likely be that the modification would completely fail for, as discussed above, AZT had no effect on the telomere length in telomerase-negative cells.

In view of the foregoing, Applicant respectfully submits that the Examiner has not established a *prima facie* case of obviousness of claims 16 and 25 under 35 U.S.C. § 103(a). Even if *prima facie* obviousness has been established, which it has not, it is urged that the cited art nonetheless fails to render the present invention obvious under a proper § 103 analysis. Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

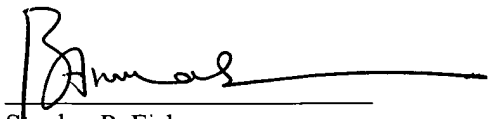
IX. Conclusion

Applicant believes this response to be a full and complete response to the Office Action. Accordingly, favorable reconsideration in view of this response and allowance of all of the

pending claims are earnestly solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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Current Perspectives on the Molecular Pathogenesis of Virus-Induced Cancers in Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

*Elliott Kieff**

A distinct group of cancers particularly threaten human immunodeficiency virus (HIV)-infected people. Most HIV/acquired immunodeficiency syndrome (AIDS)-associated cancers have a substantial component of viral etiology. Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (HHV8), human papillomavirus (HPV), and HIV have been implicated in the etiology of cancers in AIDS. The molecular mechanisms by which HPV, EBV, HHV8, and HIV persist and cause cancer are summarized. The viral etiology of AIDS-associated cancers is important because pharmacologic and immunologic strategies to prevent or attack persistent or latent virus infection and cell growth transformation may be useful in preventing and treating these cancers. Effective immune attack on latent and persistent virus infection will require enhanced cellular immune responses. Such responses may be achievable through active immunization or by *in vitro* expansion of viral and host specific cytotoxic and helper T lymphocytes. Enhanced knowledge of clinically applied T-cell immunology may also be useful in preventing and treating HIV infection and other opportunistic infections in HIV-infected people. [Monogr Natl Cancer Inst 1998;23:7–14]

Viruses are obligate intracellular parasites that are now recognized to be an etiologic factor in cancers in all human populations [reviewed in *(1,2)*]. These simple organisms have RNA or DNA genomes and have evolved to propagate themselves in human populations. Their causation of cancer is an unusual outcome of an infection that has gone awry in an individual host. Nevertheless, estimates of the frequency of virus-induced cancer worldwide are in the order of 15%–20% of all cancers. In human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS) (HIV/AIDS), viruses are estimated to cause a significantly higher fraction of cancer. The importance of viruses in HIV/AIDS-associated cancer is due to at least five factors. The first factor is the relatively young age of HIV/AIDS-infected people and the relatively low incidence of non-viral-associated cancers in young people. Second, the acquisition of HIV infection is associated with circumstances that place the individual at increased risk for acquiring other persistent virus infections. Third, some viruses that can cause persistent infections have evolved strategies for evading immune responses, for persisting in a latent state within cells, or for altering cell growth. These strategies can enable virus-infected cells to become malignant. Fourth, HIV infection alters cellular, cytokine, and humoral components of the immune response, decreases antiviral cellular immune responses, increases the burden of persistent virus infections, and thereby predisposes HIV-infected people to develop viral-associated cancers. In being at increased risk for viral-associated cancers because of decreased antiviral immune responses, HIV/AIDS-infected people are similar to other people with decreased cellular immune responses such as organ transplant recipients. Fifth, at the cellular level, HIV infection leads to provirus integration. Integrated proviral DNA can cause disruption or dysregulation of cellular gene expression and thereby initiate or promote oncogenesis. This brief overview will focus on the current status of knowledge of the molecular mechanisms by which viruses persist and effect changes in cell growth that result in cancer in patients with HIV/AIDS. Knowledge of these molecular mechanisms is highly relevant to strategies for the prevention and treatment of HIV/AIDS-associated cancers.

The viruses associated with cancer in HIV/AIDS-infected people are a subset of the viruses associated with cancer in non-HIV-infected people. In non-HIV-infected populations, human papillomaviruses (HPVs) are the initiating etiologic agents in most cervical and anogenital carcinomas, in many laryngeal carcinomas, and in a small fraction of cutaneous carcinomas. Hepatitis B and C viruses (HBV and HCV) are major factors in the etiology of hepatocellular carcinomas (HCC), a prevalent cancer worldwide. Epstein-Barr virus (EBV) is etiologically implicated in nasopharyngeal carcinoma (NPC), a cancer affecting specific populations, in B-cell proliferative disease in immune compromised patients, in lymphomas, in Hodgkin's disease (HD), and in a small fraction of gastric carcinomas. Human T-cell leukemia virus (HTLV-1) is implicated in an uncommon tumor, adult T-cell leukemia (ATL). Human herpesvirus 8 (HHV8, also known as KSHV) is implicated in Kaposi's sarcoma (KS), an unusual tumor in most human populations, but more common

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See "Note" following "References."

among some Mediterranean and African populations, in multicentric Castleman's disease, and in body cavity B-cell lymphomas (BCBLs). HPV, HBV, HCV, HHV8, and perhaps, to a small extent, HTLV-1 are more common in people exposed to blood, blood products, or sexually transmitted diseases and are therefore more prevalent in HIV/AIDS, while EBV is highly prevalent in all populations. In HIV/AIDS, HHV8 is associated with KS and BCBL, and EBV is associated with central nervous system and peripheral lymphomas, HD, and leiomyomas, all of which occur with increased frequency. In contrast, despite attributable viral etiology, HCC, cervical cancer, and ATL appear to be no more common than in the general population. HPV infection appears to be intermediate in increased association with cancer in HIV/AIDS in that while cervical and anogenital cancers are not much increased in incidence, HPV-associated premalignant lesions of all types are more common in patients with HIV/AIDS. The failure to observe an increased frequency of cervical and anogenital invasive cancers in AIDS may be due to the previously short life span of HIV-infected people and the usual long interval between HPV infection and cancer. Similarly, the long interval between HBV or HCV infection and HCC or between HTLV-1 and ATL is likely to be a factor in the low incidence of HCC and ATL in HIV/AIDS. However, the low incidence of HCC and ATL in HIV/AIDS may also be due to specific effects of HIV infection. HIV infection depletes CD4+ T lymphocytes, which are critical for enhanced CD8+ T-cell immune responses, and changes in cytokine levels shift the immune responses away from natural killer (NK) and CD8+ lymphocytes and toward B lymphocytes. Since CD8+ lymphocyte-mediated liver injury and regeneration are important in HBV or HCV induction of HCC *(3)* and interleukin 2 secretion from CD4+ cells is critical for the proliferation of HTLV-1-infected cells *(4)*, HIV infection may actually decrease the frequency of cancer associated with HBV, HCC, or HTLV-1 infection. Because HCC and ATL are not commonly associated with HIV/AIDS, they will not be further discussed in this review. Also, viral-associated benign proliferations that are important in HIV/AIDS, such as Mulluscum Contagiosum (due to the mulluscum poxvirus) and oral hairy leukoplakia (associated with EBV), are only mentioned at this point.

Human Papillomavirus

HPV 16, HPV 18, and other high-risk papillomaviruses are strongly associated with cervical, penile, and anal cancers and HPV 5 and HPV 8 have been associated with widespread epidermal, dysplastic lesions, and some carcinomas in HIV-infected and noninfected people *(5–9)*. Important aspects of the molecular mechanisms through which HPVs cause persistent infection and cancer are now partially understood. At the cellular level, HPVs infect basal epithelial cells and persist as an episome in the latently infected cell (Fig. 1). It is uncertain whether any virus-encoded proteins are expressed in infected basal epithelial cells. Basal epithelial cells replicate and some progeny differentiate into nondividing parabasal cells that further differentiate into keratinocytes. Viral replication ensues in infected differentiating keratinocytes. The HPV early promoter governs transcription of the seven HPV early genes (E1–E7). The high-risk HPV E7 proteins bind to the cellular retinoblastoma protein and thereby release the cellular transcription factor E2F. E2F is then free to

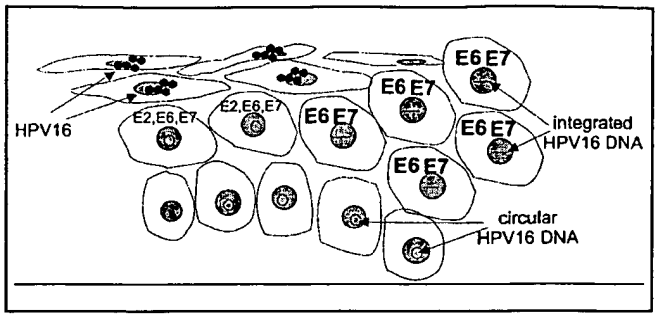

Fig. 1. Schematic depiction of HPV 16-persistent infection and replication in epithelial cells on the left with a focus of potentially oncogenic integration and high level E6 and E7 expression on the right.

up-regulate the expression of cellular genes that are important for viral and cellular DNA synthesis. High-risk HPV E6 proteins cause the degradation of p53. p53 would otherwise be induced by HPV infection, inhibit cellular cyclin dependent kinases, and cause apoptosis. The HPV E1 and E2 proteins enable viral DNA replication. When the E2 protein accumulates to high levels, the viral early promoter is down-regulated *(10)* and an as yet unidentified promoter that transcribes messenger RNAs (mRNAs) for the late viral structural proteins is presumed to be turned on.

HPV DNA integration into chromosomal DNA is not part of the normal strategy by which HPV persists in cells. Integration is the first major step away from the events of normal persistent HPV infection and toward cancer. In carcinoma tissue or carcinoma cell lines in culture, HPV DNA is usually integrated with the E2 open-reading frame interrupted so that the E2 protein is not expressed or it is unable to down-modulate the early promoter *(10–13)*. Integrations that release the HPV early promoter from the repressive effects of E2 result in continuous high-level expression of E6 and E7. While normal levels of E6 and E7 expression in an infected, differentiating keratinocyte may enable HPV to synthesize its own DNA, high-level E6 and E7 expression from a high-risk HPV in a cell that is not fully committed to terminal differentiation has immortalizing effects *(14)*. E7 has several functions, including an ability to associate with the part of the retinoblastoma protein that would otherwise bind to and inactivate E2F transcription factors *(15,16)*. The freeing of E2F results in activation of the many E2F-responsive cellular genes whose expression enables a resting cell to enter cycle, traverse G_1, and enter S phase *(17)*. In contrast to E7 that acts in the nucleus, HPV E6 acts in the cytoplasm where it binds to several cellular proteins. One important interaction is with a ubiquitin ligase *(18)*. The E6-associated ubiquitin ligase catalyzes the ubiquitination of the p53 tumor suppressor gene and its subsequent destruction by cellular proteosomes. By inducing the degradation of p53, E6 prevents p53's tumor-suppressive effects, including the induced expression of cyclin-dependent kinase (CDK) inhibitors and the induction of apoptosis. High-risk HPVs characteristically have E6 and E7 proteins that are significantly more active in targeting p53 and the retinoblastoma protein than E6 and E7 of low-risk HPVs. Although HPV DNA appears to randomly integrate into cellular chromosomes, some integration events may promote tumorigenicity by enhancing transcription of neighboring cellular oncogenes. One example is the integration of HPV DNA near c-myc in a cervical cancer cell line. c-myc is also amplified in some tumors *(11)*.

HPV infection, integration of the HPV genome into cellular DNA, and overexpression of high-risk and transforming E6 and E7 proteins are important steps in a multistep carcinogenic process. In most populations, 10%–30% of adults are infected with high-risk HPVs. HPV integration is unusual and an integration that results in HPV early promoter up-regulation is a key oncogenic event, since each tumor is marked by a single distinctive integration. Progression through low- and high-grade intraepithelial neoplasia to invasive carcinoma appears to usually require several decades. Most infected people never even progress to low-grade intraepithelial neoplasia, and invasive carcinoma is a very infrequent outcome. Many invasive carcinomas have a loss of heterozygosity at 3p, implicating this site and the FHIT gene as being important for progression *(19,20)*. Chromosome 11 has also been implicated through its activity in suppressing HPV gene expression and oncogenicity *(5)*.

One reason that most HPV-infected people never progress to invasive cervical cancer is that HPV infection can be cleared by immune mechanisms. Despite the somewhat inefficient overall immune surveillance in epithelial tissue, humoral and cell-mediated immune responses contain and eventually eliminate most HPV infections. Interferon appears to be an important component of the immune response because of its immune regulatory and antiviral activities. Nevertheless, the frequent persistence of even benign HPVs for months or years and the prolonged persistence of high-risk HPVs for decades in some people are compatible with the notion that HPV-encoded proteins may facilitate immune evasion. However, even high-risk HPVs are usually eventually cleared by the normal immune response. The immune-suppressive effects of HIV infection appear to enable longer term and higher level HPV persistence, placing HIV- and HPV-coinfected people at increased theoretic risk for cervical or anogenital cancer *(10)*. If contemporary antiretroviral therapies have their expected effects in delaying HIV disease progression and in forestalling HIV-associated mortality, HPV infections are likely to have a greater cumulative effect on cancer incidence in HIV-infected people because of the longer, at-risk exposure time. Novel strategies to prevent or treat HPV infection may be more important in this era of improved antiretroviral therapies.

Epstein-Barr Virus

EBV infection [reviewed in *(21,22)*] is similar to HPV infection in that the viral genes have a direct role in oncogenesis, but it is strikingly different in three respects. First, while the EBV genes involved in oncogenesis perform the same functions in normal latent virus infection, HPV E6 and E7 are important for early stages of viral replication and are not known to be expressed in latently infected cells. Second, high-risk E6 and E7 genes only become oncogenic as a result of integration and overexpression in cells that are not terminally differentiated. In contrast, the EBV genes important for oncogenesis are normally expressed in latent infection. They enable EBV to expand the number of latently infected cells and establish persistence in the normal host. Third, since the EBV genes associated with oncogenesis are part of the normal life cycle of the virus, their ability to cause cell proliferation must be limited so as to not cause the premature demise of the host and the virus. This later balance is struck by the reliance of the virus on the normal innate and immune response to viral infection. These responses strongly contain virus-infected cell proliferation. Because of its dependence on innate and acquired immunity, EBV infection is rarely oncogenic in people with normal immune function and is a more important cause of cancer in severely immune-compromised people, especially people with advanced HIV infection or AIDS.

EBV infection is usually spread by saliva and virus replicates in the oropharyngeal epithelium. The molecular events of intracellular replication are similar to those of other herpesviruses. In primary infection, EBV spreads from epithelial cells to B lymphocytes. In B lymphocytes, the EBV genome circularizes and expresses two nuclear proteins (EBNA-LP and -2). EBNA-LP and EBNA-2 turn on the expression of some cell genes and up-regulate their own promoter and an upstream promoter, thereby increasing transcription through the EBNA-2 polyadenylation site with downstream transcription of four additional nuclear protein (EBNA-3A, -3B, -3C, and -1) mRNAs. EBNA-LP and EBNA-2 also turn on the transcription of mRNAs encoding two integral membrane proteins (LMP-1 and -2). EBV latent infection-associated gene expression results in rather uniform progression of the infected B lymphocyte from G_0 into G_1 and S phase, the expression of small RNAs (EBERs) from the EBV genome, and continuous cell proliferation. The EBNAs and LMPs are not related to genes of other herpesvirus.

In vitro, EBV infection of B lymphocytes results in the expression of the EBNAs and LMPs and the establishment of long-term lymphoblastoid cell lines (LCLs). Similar EBV-infected LCLs result from the cultivation, *in vitro,* of peripheral blood B lymphocytes from EBV-seropositive people. Injection of EBV into cottontop tamarins causes an acute fatal polyclonal lymphoproliferative disease *(23)*, similar to that seen in severely immune-compromised humans with primary or even latent EBV infection. In more normal people, cells expressing EBNAs and LMPs engender natural killer and EBV-specific, major histocompatibility complex (MHC) class I-restricted, cytotoxic CD8+ T-cell responses *(24–26)*. The EBNAs in particular, except for EBNA-1, have multiple epitopes that are recognized in the context of common class I determinants. The EBNAs and LMP1 also induce the expression of adhesion molecules, rendering the cell susceptible to T-cell adherence and cytocidal effects. As a consequence of immune responses by normal people to primary EBV infection, the number of proliferating virus-infected B lymphocytes in the peripheral blood rapidly declines to a level of one infected B lymphocyte in 10^{-5}or 10^{-6}. However, cytotoxic T lymphocytes specific for epitopes from five of the EBNAs and the two LMPs persist forever, indicating that cells expressing the EBNAs and LMPs are at least intermittently present in the normal host.

Some EBV-infected B lymphocytes switch to a latent infection in which only EBNA-1 is expressed. These cells are apparently not proliferating and EBNA-1 is not usually recognized by immune CD8+ lymphocytes, so that cells expressing only EBNA-1 are immunologically indistinguishable from normal B lymphocytes. EBNA-1 appears to escape immune surveillance because it has a *cis*-acting glycine alanine repeat sequence that inhibits its processing through proteosomes *(27)*. The expression of EBNA-1 in the absence of other EBNAs and LMPs was first described by Rickinson et al. *(22)* in studies of Burkitt's lymphoma cell lines and tissue and has led to the working hypothesis

that there may be immunologic selection against EBV gene expression in the later stages of lymphoma evolution.

Long after primary EBV infection, EBV is closely associated with endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma, T-cell lymphomas, HD, and anaplastic NPC in normal hosts. In HD and NPC, EBNA-1 and LMPs are expressed in the absence of other EBNAs. LMP-1 appears to be a central effector of altered cell growth in lymphocytes or epithelial cells.

Recombinant EBV-based reverse genetic analyses have shown that EBNA-2, EBNA-LP, EBNA-3A, EBNA-3C, and LMP-1 are critical or essential for virus-mediated B-cell growth transformation *(28)*. EBNA-1 is also presumed to be important, since EBNA-1 binds to a site upstream of the EBNA promoter, thereby enhancing transcription and creating a functional origin for replication of the EBV episome in cellular S phase *(29)*. LMP-2, EBNA-3B, small non-polyadenylated RNAs (EBERs), and a long highly spliced mRNA (BARF0), which are also expressed in latent lymphocyte infection, are unimportant for primary B-lymphocyte growth transformation. Their role in latent EBV infection is uncertain except for LMP-2. LMP-2 has a critical role in rendering the transformed cell not susceptible to activation signals that would otherwise result in the activation of lytic EBV infection. Most of the rest of the viral genome, including EBV-encoded IL10 and Bcl2 analogues that are ordinarily expressed in lytic infection, have also formally been shown to be unimportant in the conversion of resting B lymphocytes into LCLs and subsequent growth into tumors in SCID mice.

Recent biochemical and reverse genetic analyses are compatible with the hypothesis that most of EBV's effects on cell growth are mediated by virus-encoded proteins that usurp control of the notch and CD40 signaling pathways. Early biochemical work established EBNA-2 as a transactivator of expression of the B-lymphocyte activation marker, CD23, and of LMP-1 transcription. Subsequent recombinant EBV reverse genetic analyses defined three critical components of the EBNA-2 open-reading frame. The first is simply several codons that encode prolines near the amino terminus of EBNA-2. These codons are the core of a dimerization element. The second component is codons 280–337 that mediate interactions with PU.1, a B lymphocyte and macrophage-specific "ets" family transcription factor, and with RBP-Jk, a transcription factor that has been genetically implicated in notch-mediated neural development in *Drosophila*. EBNA-2 in an EBV-transformed lymphoblast is strongly associated with RBP-Jk and a substantial fraction of the cellular RBP-Jk is associated with EBNA-2 *(30)*. The third essential EBNA-2 component is codons 424–464, which encode an acidic activator. This acidic activator can interact with TFIIB, TAF40, and two subunits of TFIIH and extensively associates with a novel coactivating protein, p100, and with citric acid lyase, a major acetyl-CoA donor. P100 can interact with two subunits of TFIIE. P100 also has a domain that is functionally equivalent to the major carboxyl terminal negative regulatory domain of the c-myb protein *(31)*. Recent experiments indicate that EBNA-LP coactivates transcription along with EBNA-2. EBNA-LP is not an independent transactivator, but the localization of the EBNA-2 acidic domain near a promoter enables EBNA-LP to strongly coactivate transcription from that promoter *(32)*.

Surprisingly, EBNA-3A and EBNA-3C (as well as EBNA-3B, which is not critical for growth transformation) also associate with RBP-Jk and regulate expression of specific virus and cell genes with RBP-Jk sites. The central role of RBP-Jk in EBV regulation of viral and cellular gene expression, evidence that notch activation is a cause of T-cell leukemias *(33)*, and evidence that RBP-Jk mediates notch effects, are all compatible with the hypothesis that RBP-Jk may mediate the regulation of transcription of some cellular gene(s) that are important in lymphocyte growth control.

EBV uses the EBNAs and RBP-Jk to precisely regulate transcription of its oncogene, LMP-1 (Fig. 2). LMP-1 expression in Rat1 cells causes loss of contact inhibition, anchorage-independent growth, and tumorigenicity in nude mice. In lymphocytes, LMP-1 enhances bcl-2 expression, activates NF-kB and c-jun, and induces most of the activation and adhesion molecules that are activated by EBV infection or by antigen and T-cell help. T cells express CD40 ligand, and the T-cell CD40 ligand/B cell CD40 receptor complex is a key component of T-cell help.

LMP-1 has six hydrophobic transmembrane domains that enable it to constitutively aggregate in the plasma membrane and are essential for EBV transformation of B cells into LCLs (Fig. 3). The observation that a mutation in the LMP-1 transmembrane domains that results in diffuse expression in the plasma membrane has a nontransforming phenotype indicates that aggregation is linked to transformation, most likely because aggregation enables the cytoplasmic domains of several LMP1 molecules to locally concentrate next to the plasma membrane as though they were the cytoplasmic domains of a growth factor receptor that had encountered ligand. The LMP1 amino terminal cytoplasmic domain is not critical, but the carboxyl terminal cytoplasmic domain has two important components: a proximal component that is sufficient for initial immortalization (transformation effector site 1, TES1) and a distal domain that enables efficient LCL outgrowth (TES2) *(28,34,35)*. TES1 and TES2 mediate 30% and 70% of the NF-kB activation from LMP-1, respectively. Since TES1 only mediates a small component of NF-kB activation, but is both necessary and sufficient for initial lymphocyte immortalization, NF-kB can only be a component of TES1 LMP1-mediated growth transformation. Two proteins that interact with LMP1 were identified in a yeast-two hybrid screen and in a screen for proteins induced by EBV infection. These proteins are highly homologous to proteins identified as mouse tumor necrosis factor receptor II (TNFRII)-associated factors or

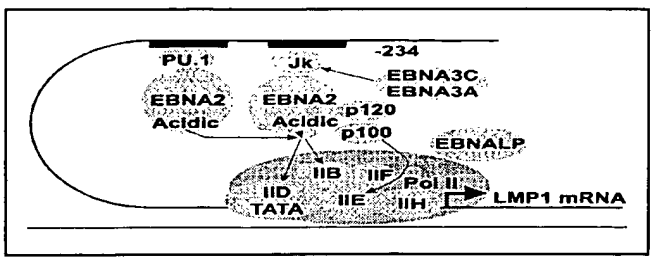

Fig. 2. Schematic diagram of the regulation of the EBV LMP1 promoter by EBV nuclear proteins in latently infected B lymphocytes. Not shown is EBNA-1 that also contributes to LMP1 up-regulation through its binding to the ori-p element, which is separated by about 10 kilobase pairs from the LMP1 promoter.

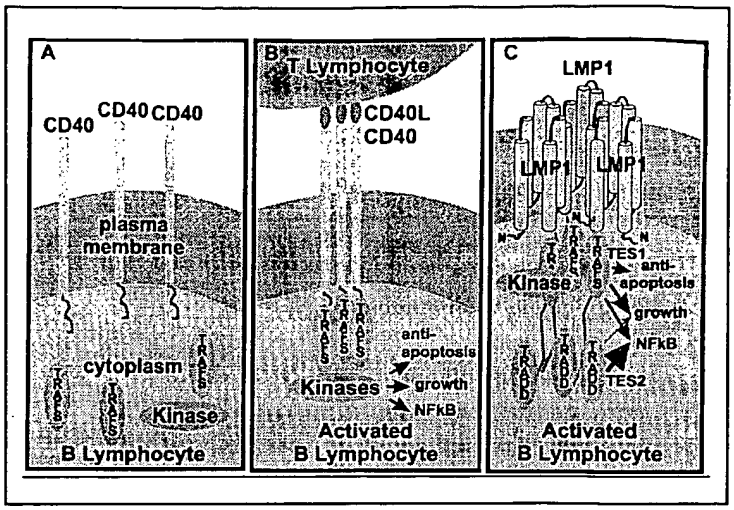

Fig. 3. Schematic diagram of signal transduction from EBV LMP1 and its similarity to ligand-activated CD40 and other activated tumor necrosis factor receptors. LMP1 is constitutively activated because of the six hydrophobic transmembrane domains that cause LMP1 to noncovalently aggregate in the plasma membrane.

TRAFs. LMP1 is similar to activated CD40 in its effects on B-lymphocyte growth and CD40 is also a TNFR that associates with TRAFs, implicating TRAFs in signaling from LMP1 (Fig. 3). In EBV-transformed B lymphoctes, LMP1 is constitutively strongly associated with TRAF1 and TRAF3 and is also associated to a lesser extent with TRAF2 *(36)*. Such cells have a high level of TRAF1/2 heterodimers that mediate NF-kB activation from TES1. The pathway appears to be similar to TNFRII, LT-beta receptor, or CD40 ligand-dependent activation of NF-kB through TRAF2. Furthermore, like CD40, LMP1 activates SAPK and c-jun through TRAF2. Thus, the LMP-1 transmembrane domains mediate constitutive aggregation in the plasma membrane, enabling the LMP1 cytoplasmic domain to be associated with TRAFs and mediate NF-kB and c-jun activation, as well as other less well-characterized effects important for cell growth stimulation. Deletion of DNA encoding the TRAF binding site from the LMP1 gene in recombinant EBVs results in a null phenotype for resting B-lymphocyte growth transformation.

Recent experiments *(35)* indicate that TES2 maps to the last three residues of LMP1. High-level NF-kB activation is also mediated by this site, providing genetic evidence that high-level NF-kB activation is important in LMP1's effects. The TNF receptor death domain protein (TRADD) uniquely interacts with TES2 and is constitutively associated with LMP1 in EBV-transformed B lymphocytes. These data implicate TRADD in LMP1-mediated growth transformation and NF-kB activation, underscoring the degree to which LMP1 mimics a constitutively activated TNFR. Curiously, LMP1 engagement of TRADD does not appear to effect cell death, whereas bcl-2 and NF-kB activation by LMP1 protect EBV-infected cells from cell death.

Most of the central nervous system lymphomas that occur late in AIDS and some of the peripheral lymphomas are characterized by the expression of the full range of EBNAs and LMPs. The occurrence of EBV-associated lymphomas is consistent with earlier studies, indicating that the number of lymphocytes latently infected with EBV increases during HIV infection. Waning cytotoxic T-cell immunity is likely to be a critical factor in the increased abundance of EBV-infected cells in the peripheral blood and the subsequent emergence of lymphoma. Changes in cytokine levels and the shift in cytokine balance toward B lymphocyte up-regulation may also have a role.

The fact that many of the EBV-associated lymphomas that occur late in AIDS are similar in viral and cellular gene expression to resting B lymphocytes transformed by EBV infection *in vitro* and to B lymphocytes in the EBV-associated oligoclonal lymphoproliferative disease that can occur with primary EBV infection in organ transplant recipients who are receiving high dose immune-suppressive therapy has two important implications. First, the lymphoma cells may still be critically dependent on EBV gene expression for their proliferation. Second, the ability of cytotoxic T lymphocytes from normal EBV-infected people to kill latently infected cells through EBNA- or LMP-derived epitopes in the context of common class I MHC molecules can be exploited for the prevention or treatment of EBV-induced lymphoproliferative diseases in which these viral proteins are expressed.

Clinical research with human T-cell immunotherapy has already achieved some promising results in post-transplant patients *(37,38)*. For bone marrow transplant recipients, infusion of donor peripheral blood mononuclear cells or of donor T-cell lines derived through exposure of T lymphocytes to autologous EBV-transformed B lymphocytes has resulted in the regression or the prevention of EBV-induced lymphoproliferative disease. In initial studies, donor T cells appear able to survive, hone to sites of proliferating EBV-infected cells, effect killing, and proliferate to a limited extent. Similar approaches might be beneficial in patients with AIDS. Since the majority of patients with AIDS escape EBV-associated lymphomas, efforts to increase cytotoxic T-cell responses by active immunization or by expansion of EBV-reactive autologous T lymphocytes, *in vitro,* and reinfusion might confer protection against EBV-associated lymphoproliferations.

Many EBV-associated cancers in patients with HIV/AIDS are likely to be EBV-initiated and to have subsequent chromosomal changes that advance the malignant phenotype. Subsequent chromosomal changes are well described in Burkitt's lymphomas and also characterize much of the spectrum of lymphoproliferative disease that occurs in post-transplant lymphomas and HIV/AIDS *(39)*. The most frequent change is a c-myc translocation leading to dysregulated c-myc expression. Activation of c-myc does not necessarily indicate independence of EBV gene expression for continued cell growth, and EBV gene expression

in malignant cells provides a basis for immune attack on the tumor cell.

Other EBV-associated cancers that occur with a higher frequency in HIV/AIDS include HD and leiomyosarcomas. EBV-associated HD is characterized by EBNA-1, LMP2, and high-level LMP1 expression and by the absence of expression of other EBNAs. With regard to HD, the similarities of LMP1's biochemical effects to those of activated CD30, the high level of CD30 expression on EBV-associated as well as EBV non-associated HD tumor cells, and the presence of T cells that are likely to express CD30 ligand in HD tissues are compatible with the hypothesis that CD30 and LMP1 are parallel signaling pathways important in HD tumor cell growth. EBV-infected HD cells may also be subject to immune attack *(40)*. While lymphomas and leiomyomas are substantially increased in patients with HIV/AIDS and HD is marginally increased, NPC, the most common EBV-associated cancer worldwide, is uncommon in patients with AIDS. As with cervical cancer and HCC, the failure to observe an increased incidence of NPC in HIV/AIDS may be related to the long interval between EBV infection and NPC.

HHV8 (KSHV)

The discovery of HHV8 (or KSHV), a second human gamma herpesvirus, in human KS tissue by Chang et al. *(41)* is a breakthrough in understanding the etiology of KS. Previously, cytokines and HIV TAT had been implicated in the genesis of KS *(42)*. However, TAT levels have been difficult to measure in tissues and the epidemiology of KS in HIV-infected people is most compatible with a second sexually transmitted agent *(43)*. Importantly, KS tumors from patients with HIV/AIDS or even from patients with familial KS almost always contain HHV8 DNA *(44)*. Furthermore, KS spindle cells that express CD54, an endothelial cell marker, also have RNA from a restricted portion of the HHV8 genome, indicating that these cells are latently infected with HHV8 *(45)*. A few spindle cells also contain RNA encoding a late viral structural protein, which is compatible with similar findings in EBV-associated lymphoproliferative lesions. Moreover, in patients with HIV/AIDS, antibody to KSHV is a predictor of subsequent KS. Although there has been considerable controversy about the frequency of HHV8 DNA in semen and peripheral blood and about the frequency of HHV8 antibody in various populations, more recent data are consistent with the notion that HHV8 is a sexually transmitted virus that may infect 10% of the general population and a higher fraction of the HIV/AIDS population *(46,47)*. Thus, there is now considerable evidence implicating HHV8 in KS. However, relatively little is known about the mechanisms by which HHV8 might effect cell growth. Additional evidence that would strengthen the case for HHV8 in the etiology of KS and BCBLs *(48)* could come from studies demonstrating that HHV8 can transform cells in culture or induce tumors in heterologous species.

The etiology of BCBL may be complex. An HHV8 ORF73-encoded viral nuclear protein is expressed in BCBL cells *(49)*. The ORF73 latency-associated HHV8 protein has been termed LANA for latency-associated nuclear antigen. However, EBV is also usually present in BCBLs and may have a role in oncogenicity.

BCBL cells can be grown in culture and EBV-free BCBLs are at the present time the best source of HHV8 DNA or proteins. HHV8 replication can be induced in BCBL cells and large quantities of HHV8 are produced from some cell lines *(50)*. So far, only limited replication has been described in primary B lymphocytes and only abortive infection has been described in 293 cells.

Sequence analysis of the HHV8 genome has revealed homology to other herpesviruses, particularly to the rhadinovirus subgroup of the gamma herpesviruses. While EBV has been the most intensely studied gamma herpesvirus, much is also known about the somewhat more closely related rhadinovirus, herpesvirus *saimiri* (HVS). HVS is a T-lymphotropic gamma herpesvirus that can induce T-cell lymphomas in some heterologous species, but has no such effect on its natural host *(51,52)*. HVS has been investigated using biochemical and molecular genetic approaches and may offer some instructive precedent for how HHV8 may cause persistent infection and effect cell growth (Fig. 4). HVS has two putative transforming genes near the left end of its genome. The H. *saimiri* transforming protein and tyrosine kinase interacting protein encoding genes, STP and TIP, vary among HVS strains. Recombinant HVS reverse genetic analyses indicate that STP and TIP are essential for HVS lymphomagenesis. STP up-regulates the Ras pathway *(53)*, whereas TIP down-regulates Lck and activates NF-kB *(54)*. TIP down-regulation of Lck appears to be unimportant for transformation, while TIP-mediated NF-kB activation is likely to be important in lymphomagenesis. Whether the HVS-encoded cyclin homolog, a superantigen encoding open-reading frame, a bcl-2 homolog, and an IL-17 homolog have any role in HVS-induced lymphomagenesis is uncertain. HVS also has several open-reading frames that are likely to be important in escape from immune surveillance and similar open-reading frames are present in HHV8.

HHV8 has 75 open-reading frames that are homologous to HVS, which have been designated ORF1-ORF75, and at least 15 novel open-reading frames that have been designated K1–15 *(51)*. K1 is positioned at the site of HVS STP and TIP and is also variable in sequence among HHV8 strains. Two other novel HHV8 genes could also be important in transformation. HHV8 K2 encodes a functional IL-6 homolog that could be important in endothelial cell growth and HHV8 K13 encodes an inhibitor of FADD-activated apoptosis *(52,55–58)*. Other HHV8 open-reading frames that have homologs in HVS of unproven significance for HVS-induced T-cell transformation are HHV8 ORF72, the cyclin homolog, and ORF16, a bcl-2 homolog. However, the only HHV8 protein known to be expressed in latently infected cells is the ORF73-encoded protein, LANA. A

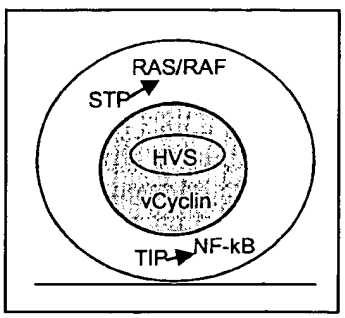

Fig. 4. Schematic diagram of the molecular mechanisms by which herpesvirus *saimiri* (HVS) induces T-cell lymphomas in heterologous primate species. Genetic analyses have confirmed the importance of STP and TIP in T-cell lymphomagenesis. The role of the HVS cyclin D homolog in T-cell lymphomagenesis has not been evaluated, because the gene is essential for virus replication.

lytic infection-associated protein such as the HHV8 IL-6 homolog could be important for KS endothelial cell growth if it is expressed in large amounts from lytically infected cells and such cells are sufficiently prevalent to sustain high cytokine levels. Persistent lytic poxvirus infections, for example, cause hypertrophic cutaneous lesions through secretion of epidermal cell growth factor-related and immune modulating factors. Poxviruses that cause hypertrophic lesions appear to be unique in that infection is highly localized, all infected cells are persistently lytically infected, and there is almost a complete blockade of an effective immune response.

Human Immunodeficiency Virus

The frequency with which HIV itself causes cancer is uncertain and only limited data are available. The positive data are from analyses of non-B-cell lymphomas of all types that were HIV p24 positive *(59)*. Of 22 specimens analyzed in two studies, 18 were positive for HIV proviral DNA by inverse polymerase chain reaction. Of the 18 cases in which proviral DNA was detected, 10 were integrations of HIV upstream of the c-fes gene. Proviral integration upstream of c-fes was found in one T-cell lymphoma. However, almost all of the other integrations were in macrophage-like cells and not in the tumor cells. These findings have led to the hypothesis that HIV integration near c-fes might result in up-regulation of a cytokine that might contribute to lymphomagenesis. Given the frequent finding of defective proviral integrations in HIV infection overall and the substantial activity of the HIV promoter, particularly in cells with high basal NF-kB activation, HIV may be a more significant factor in HIV/AIDS-associated cancer than is currently appreciated.

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